

QUANTITATIVE EXPERIMENTS ON THE
NUTRITIVE VALUE OF AMINO ACIDS

HAINES BOOTS LOCKHART

A. B., Wabash College, 1942

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY
IN THE GRADUATE SCHOOL OF THE
UNIVERSITY OF ILLINOIS, 1945

URBANA, ILLINOIS

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ACKNOWLEDGEMENT

The author wishes to express his sincere appreciation to Professor William C. Rose for his suggestion of this problem and for his invaluable assistance and advice throughout this research.



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PART I

AN ATTEMPT TO DEVISE A BIOLOGICAL METHOD FOR THE ESTIMATION OF VALINE IN PROTEINS

INTRODUCTION

Although numerous methods are given in the literature for the determination of the amino acid content of proteins, the procedures for the estimation of valine are not only few and difficult to carry out, but the results leave much to be desired. It would be to the advantage of nutritionists and other students of protein chemistry if some accurate method for the determination of this amino acid were discovered.

Bonner and Rose¹ using rats as experimental animals developed a procedure for the biological estimation of threonine. Since these investigators obtained satisfactory results with this method, it was decided to apply the biological technique to the quantitative estimation of valine.

EXPERIMENTAL

Twenty-three litters of young albino rats weighing from 40 to 60 grams each were maintained on valine-free purified amino acid diets for 12 days. After this control period, a group of the subjects were placed on diets containing from 0.10 to 0.70 per cent of 1(+)-valine. The duration of the experimental period was 16 days. From the data obtained with the rats on this regime, a curve was prepared indicating the effects of valine increments upon the growth rate. The remainder of the animals were given food in which all of the 1(+)-valine was supplied by known percentages of the following proteins: casein, glycinin, gliadin, peanut protein, blood fibrin, lactalbumin, zein, and oxyhemoglobin. The level at which a protein was fed was selected so as to give growth which fell within a satisfactory

region on the 1(+)-valine growth curve. By comparing the results obtained with rats on (1) the 1(+)-valine diets and (2) the protein-supplemented diets, it was anticipated that it would be possible to estimate the amount of this amino acid which was furnished to the diet by the protein. Under these conditions, the per cent of 1(+)-valine *in the protein* would be the product of the per cent of the amino acid supplied by the protein and a factor found by dividing 100 by the per cent of the protein in the diet.

The 1(+)-valine content of several proteins fed at a 5 per cent level as determined by this method were found to be: casein, 4.5 per cent; glycinin, 3.1 per cent; gliadin, 2.4 per cent; blood fibrin, 3.1 per cent; peanut protein, 3.0 per cent; lactalbumin, 3.3 per cent; zein, 2.4 per cent; and oxyhemoglobin, 6.5 per cent. However, the value obtained when 8 per cent of casein was fed was 8.0 per cent. This appears to demonstrate that factors other than the valine content of the supplemental protein may affect the growth of the animals, and thus casts doubt upon the validity of figures representing the percentage of valine in the protein.

SUMMARY

An attempt was made to determine the 1(+)-valine content of eight proteins by use of a biological method. The values obtained are given above.

The results were not so consistent as was hoped, particularly with diets containing casein. Under the circumstances, one is obliged to assume the growth-stimulating action of certain proteins, when incorporated in a valine-free diet, is not due solely to the quantity of valine furnished by the protein.

BIBLIOGRAPHY

- ¹ Bonner, P., and Rose, W. C., Unpublished data.

PART II

THE VALINE AND THREONINE REQUIREMENTS FOR THE MAINTENANCE OF NITROGEN EQUILIBRIUM IN MAN

INTRODUCTION

It has been shown by nitrogen balance experiments conducted in this laboratory that only eight amino acids are required for the maintenance of nitrogen equilibrium in man^{1,2,3}. These are lysine, phenylalanine, tryptophane, leucine, isoleucine, threonine, methionine, and valine. The quantitative requirements for tryptophane⁴ and lysine⁵ have already been established. The recommended daily intakes of these two amino acids are 250 and 600 mg., respectively. In the course of these quantitative experiments it was noted that man cannot utilize the unnatural form of either tryptophane or lysine.

In the present investigation the quantitative human requirements for valine and threonine were investigated, and the unnatural isomers were tested. Since Holt, *et al.*,⁶ have reported the presence in the urine of histidine-deficient subjects of a metabolite which gives a green pigment when measured as indican according to the Sharlit procedure⁷, quantitative studies of indican were also made.

EXPERIMENTAL

Normal adult male subjects were used in these experiments. The diets contained 10 grams of nitrogen, of which over ninety-five per cent was supplied in the form of purified amino acids and urea. The other constituents of the rations were corn starch, sucrose, butter, cellu-flour, corn oil, minerals, lemon juice, haliver oil concentrate, and a mixture of vitamins. Distilled water was taken *ad libitum*. It was found that in order to maintain a subject in positive nitrogen balance, the total caloric intake should exceed 2000 calories per square meter of body surface. Since it was also noted that the ratio of calories in the form of carbohydrate to calories in the form of fat in the diet should be greater than 2, the value of 2.6 was used.

Twenty-four hour urine samples were collected and preserved with toluene. Analyses were made daily for total nitrogen, creatinine, and indican. The feces for each dietary period were separated by charcoal markers. They were dried, mixed well, and an aliquot was analyzed for total nitrogen.

Each subject was placed on a control diet containing an ample amount of the amino acid under investigation until nitrogen balance was established. In subsequent periods, the concentration of the amino acid was decreased until negative nitrogen balance developed. At this point, the level of the amino acid was increased until positive balance was attained. This procedure was followed until a response to a change of 0.1 gram per day of the natural isomer was observed. The minimum daily requirement is defined as *the lowest level of an amino acid necessary to maintain consistent positive nitrogen balance.*

Using this criterion, the minimum daily level of 1(+)-valine was established as 800 mg. The requirement of the human organism for d(—)-threonine, as determined with two subjects, was 300 and 400 mg., respectively. To allow for such individual variations, the recommended intake for 1(+)-valine is 1.20 grams, while that of d(—)-threonine is 0.60 gram. The unnatural optical isomers of these amino acids cannot replace the natural isomers on an equal weight basis. The color produced by the metabolite in the urine did not check that reported by Holt, *et al.* Also, there was no correlation between the output of this compound and the intake of histidine.

SUMMARY

The requirements of the human organism for valine and threonine have been determined. These values, as well as the recommended daily intake of these amino acids, are given above. The inability of man to utilize the unnatural isomers of these amino acids in place of the natural forms has been established. The concentration of histidine in the diet has no effect on either the amount or the color of the metabolite in the urine measured as indican.

BIBLIOGRAPHY

- ¹ Rose, W. C., Haines, W. J., and Johnson, J. E., *J. Biol. Chem.*, **146**, 683 (1942).
- ² Rose, W. C., Haines, W. J., Johnson, J. E., and Warner, D. T., *J. Biol. Chem.*, **148**, 457 (1943).
- ³ Rose, W. C., *Proc. Inst. Med.*, **15**, 24 (1944).
- ⁴ Lambert, G. F., and Rose, W. C., Unpublished data.
- ⁵ Borman, A., and Rose, W. C., Unpublished data.
- ⁶ Albanese, A. A., Holt, L. E., Jr., Frankston, J. E., and Irby, V., *Bull. Johns Hopkins Hosp.*, **74**, 251 (1944).
- ⁷ Sharlit, H., *J. Biol. Chem.*, **99**, 537 (1932-33).

VITA

The author was born in Crawfordsville, Indiana, on October 29, 1920. He attended Mill's Grade School and Crawfordsville High School, graduating in 1938. He entered Wabash College the same year and received an Honor Scholarship for the four years he attended the college. His A.B. degree was received in June, 1942. The fall of that year, the author entered the Graduate School of the University of Illinois as a Teaching Assistant. A few months later, he received a half-time special Research Assistantship which he retained for the remainder of the period in which he was doing graduate study.

